

US006417335B1

(12) United States Patent Basey et al.

(10) Patent No.: US 6,417,335 B1

(45) **Date of Patent:** Jul. 9, 2002

(54) **PROTEIN PURIFICATION**

(75) Inventors: Carol D. Basey, Winters; Greg S. Blank, Menlo Park, both of CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

- (21) Appl. No.: 09/680,148
- (22) Filed: Oct. 3, 2000

Related U.S. Application Data

- (62) Division of application No. 09/304,465, filed on May 3, 1999.
- (60) Provisional application No. 60/084,459, filed on May 6, 1998.
- (51) **Int. Cl.**⁷ **C07K** 1/18; C07K 16/00

(56) References Cited

U.S. PATENT DOCUMENTS

5,110,913 A	* 4	5/1992	Coan et al	530/416
5,112,951 A	* 1	5/1992	Beidler et al	530/416
5,118,796 A	* 4	6/1992	Prior et al	530/416
5.821.337 A	4	10/1998	Carter et al.	

FOREIGN PATENT DOCUMENTS

EP	333574	9/1989
EP	556083	8/1993
EP	460426 B1	9/1997
WO	WO 89/05157	6/1989
WO	WO 96/33208	10/1996

OTHER PUBLICATIONS

Carter et al., "Humanization of an anti–p185^{HER2} antibody for human cancer therapy" *Proc. Natl. Acad. Sci.* 89:4285–4289 (May 1992).

Gagnon, P., "Ion Exchange Chromatography" *Purification Tools for Monoclonal Antibodies*, Tucson: Validated Biosystems, Inc., Chapter 4, pp. 57–86 (1996).

Graf et al., "Ion exchange resins for the purification of monoclonal antibodies from animal cell culture" *Bioseparation* 4 (1):7–20 (Feb. 1994).

Harris, R., "Chromatographic Techniques for the Characterization of Human MAbs" (Slides presented at the Waterside Monoclonal Conference held at the Omni Waterside Hotel in Harborside–Norfolk, Virginia on Apr. 22–25, 1996) pp. 1–7. Mhatre et al., "Purification of antibody Fab fragments by cation–exchange chromatography and pH gradient elution" *Journal of Chromatography A* 707 (2):225–231 (Jul. 21, 1995).

Neidhardt et al., "Rapid, two-step purification process for the preparation of pyrogen-free murine immunoglobulin G_1 monoclonal antibodies" *Journal of Chromatography* 590 (2) :255-261 (1992).

Sofer et al. *Handbook of Process Chromatography: A Guide to Optimization, Scale-up, and Validation*, San Diego:Academic Press pp. 65–80 (1997).

Tishchenko et al., "Effect of salt concentration gradient on separation of different types of specific immunoglobulins by ion–exchange chromatography on DEAE cellulose" *Journal of Chromatography B* 706 (1):157–166 (Feb. 27, 1998).

* cited by examiner

Primary Examiner—David Saunders (74) Attorney, Agent, or Firm—Wendy M. Lee

(57) ABSTRACT

A method for purifying a polypeptide by ion exchange chromatography is described which involves changing the conductivity and/or pH of buffers in order to resolve a polypeptide of interest from one or more contaminants.

9 Claims, 7 Drawing Sheets

